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PHARMACOGNOSTIC ANALYSIS AND PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF EUPHORBIA HIRTA LEAVES.

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ABSTRACT

Medicinal plants are oldest known health care products and their importance is still growing. Globally demand for *swadesi* herbal medicines has been rising due to their quality ingredients, availability factor and price competitiveness with virtually little side effects. Increasingly, *swadesi* herbs and medicines meet the WHO prescribed standards and norms and thus encounter no restrictions in overseas markets to have instant acceptability from its takers. Several studies are needed to be conducted to develop the phytochemical and pharmacognostic profile of plant medicine based on which its pharmacological screening of plant extracts can be designed to develop new plant medicines or lead molecules. Hence the objective of the present study was to assess the pharmacognostic parameters of *Euphorbia hirta* leaves to determine its quality and then followed by assessing the organoleptic and phytochemical nature of the methanolic extracts of Euphorbia hirta leaves. The selected plant part was dried, powdered and subjected to maceration using methanol as solvent. The extract was dried for evaporation of solvent and then subjected to organoleptic and phytochemical studies. The results of pharmacognostic studies revealed the physicochemical parameters of *Euphorbia hirta* leaves. The phytochemical studies indicate that the methanolic extract showed presence of alkaloids, carbohydrates, flavonoids, tannins, triterpenoids and steroids.

KEYWORDS: *Euphorbia hirta* leaves, methanolic extract, pharmacognostic studies and phytochemical analysis.

Introduction:

Euphorbia hirta is a pantropical weed, possibly native to India. It is a hairy herb that grows in open grasslands, roadsides and pathways. It is widely used as a medicinal herb. *Euphorbia hirta* is an annual growing to 0.3 m (1ft) by 0.3 m (1ft in). It is frost tender. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by Insects.Suitable for: light (sandy) and medium (loamy) soils and prefers well-drained soil. Suitable pH: acid, neutral and basic (alkaline) soils. It cannot grow in the shade. It prefers dry or moist soil.[1,2]

*Euphorbia hirta*L. is a medicinal, rhizomatous herb distributed in Southern Western Ghats of India and Northern East Coast of Tamil Nadu. C. In East and West Africa extracts of the plant are used in treatment of asthma and respiratory tract inflammations. It is also used for coughs, chronic bronchitis and other pulmonary disorders in Malagasy. The plant is also widely used in Angola against diarrhoea and dysentery, especially amoebic dysentery. In Nigeria extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing.[3]

The plant has been used for female disorders but is now more important in treating respiratory ailments, especially cough, coryza, bronchitis and asthma. In India it is used to treat worm infestations in children and for dysentery, gonorrhoea, jaundice, pimples, digestive problems and tumours.[4]

Materials and methods:

Plant Materials: Leaves of *Euphorbia hirta*s were collected from local region of Tirupati and was authenticated by Head of the Department, Department of Botany, S.V Arts College, Tirupati.

Chemicals: Methanol, alcohol, α- naphthol, concentrated sulphuricacid, dilutehydrochloric acid, glacial acetic acid, acetone, choloroform, and Distilled water used were analytical grade.

Pharmacognostic Analysis:

The pharmacognotic analysis of dried sample of *Euphorbia hirta* leaves were carried out as follows.

Physicochemical parameters:

Physiochemical values like the percentage of ash values and extractive values etc., were determined according to the official methods [3,4] and as per WHO guidelines on quality control methods for medicinal plant materials[5,6].

Water soluble extractive value of *Euphorbia hirta*s leaves:

About 5 g of powdered *Euphorbia hirta*s leaves was added to 50 ml of water at 80°C in a stoppered flask. It was shaken well and allowed to stand for 10 minutes. It is then cooled to 15°C followed by addition of 2g of kieselghur and filtered. 5 ml of filtrate was transferred to tarred evaporating basins and evaporated on a water bath and the residue was weighed. The percentage of water soluble extractive was calculated with reference to one gram of dried sample of plant extact.[20]

Alcohol soluble extractive value of *Euphorbia hirta*s leaves:

About 5 g of powdered *Euphorbia hirta*s leaves was macerated with 100 ml of 90% ethanol in a closed flask for 24 h, shaking frequently during 6 h and allowed to stand for 18 h. It was filtered immediately taking precaution against loss of alcohol and 25 ml of filtrate was evaporated to dryness in a tarred flat bottomed shallow dish and dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to one gram of dried sample of *Euphorbia hirta* leaves.[20]

Loss on drying of *Euphorbia hirta*s leaves:

About 1 gm of powdered *Euphorbia hirta*s leaves was transferred into a petridish plates and the content was distributed evenly to a depth not exceeding 10 mm. The loaded plate was heated at 105°C in hot air oven for 1 hr and then cooled in desiccator, loss in weight was recorded as moisture content or loss on drying. The moisture content percentage of the sample was calculated.

$$\text{Formula:} \quad \frac{w'_2 - w_3}{w_2 - w_1\,(100-H)} \times 100$$

W_1 = Empty Petri-dish weigh,

w_2 = Petri-dish + sample weight,

w_3=Petri-dish + sample weight after oven,

w'_3 = Weight after desiccate, H = loss on drying

Total ash value of *Euphorbia hirta*s leaves:

The total ash was determined by incinerating 2 g of powdered *Euphorbia hirta*s leaves in a tarred silica crucible which was previously ignited and cooled before weighing. The drug was incinerated by gradually increasing the heat in a muffle furnace at 450°C for 4 hrs. Till the constant weight was obtained ignition was repeated. After complete incineration, it was cooled in a desiccator. Then the percentage of the total ash with reference to per gram of dried sample of *Euphorbia hirta*s leaves was calculated.[20]

$$\text{Formula:} \quad \frac{w'_3 - w_1}{w_2 - w_1\,(100-H)} \times 100$$

w_1 = Empty crucible weight,

w_2 = Crucible + sample weight,

w_3 = Crucible + sample weight after burning,

w'_3 = Weight after desiccate, H = Loss on drying

Acid – insoluble ash of *Euphorbia hirta*s leaves:

The ash of powdered *Euphorbia hirta*s leaves was washed from the crucible into 100 ml beaker using 25 ml of 2 N HCl. Then boiled for 5 min over a Bunsen

burner and filtered through an ashless filter paper (Whatman No: 42).Using hot water the residue was washed twice, ignited to ash, cooled in desiccator and weighed. The residue was weighed and the acid insoluble ash of the drug was calculated with reference to the dried sample of *Euphorbia hirta*s leaves.[20]

$$\text{Formula:} \quad \frac{w'_4 - w_1}{w_2 - w_1\,(100-H)} \times 100$$

w_1 = Empty crucible weight,

w_2 = Crucible + sample weight

w_3 = Crucible + sample weight after burning,

w_4 = Burn filter paper + crucible weight,

w'_4 = Weight after desiccate,

H = Loss on drying

Foreign matter in *Euphorbia hirta*s leaves:
About 250 g of powdered *Euphorbia hirta*sleaves was weighed. It was spread in a thin layer and foreign matter was sorted into groups with the help of a sieve. Sifted the remainder of the sample through a No. 250 sieve. 0.05 g of sorted foreign matter was weighed. Calculated the content of each group in grams per 100 g of air-dried sample.[5]

Preparation of extracts:
To prepare the methanolic extract, 150 g of *Euphorbia hirta*s leaves was collected, air driedand reduced to powder. It was macerated with methanol and was allowed to stand for 72 hrs at room temperature and then filtered. The filtrate was then evaporated under reduced pressure and dried using a rotary evaporator at 55°C. Dried extract was stored in labeled sterile screw capped bottles at 5°C in the refrigerator.[9]The prepared extracts were used for Organoleptic characterization, and screening of phytochemical parameters.

Phytochemical analysis:
Preliminary phytochemical screening:
Plants are considered as bioreactors or biosynthetic laboratories as they synthesize wide range secondary metabolites which aretherapeutically important. Thus, to establish a chemical profile of a crude drug for its proper evaluation a systematic preliminary phytochemical screening of plant material is essential for identifying plant constituents. Phytochemical screening of the methanolic extract of *Euphorbia hirta*s leaves was carried as per systematic methods.[7,8].

Test for Carbohydrates:
Molisch's test: To 2 ml of methanolic extract of *Euphorbia hirta*, two drops of alcoholic solution of α- naphthol were added. The mixture was shaken well and few drops of concentrated sulphuric acid was added slowly along the sides of test tube.[10]

Fehling's test: In a test tube 2 ml of methanolic extract of *Euphorbia hirta*was taken and equal volumes of Fehling A & Fehling B solutions were added and placed it in a boiling water bath for few minutes.[10]

Benedict's test: To 0.5 ml of methanolic extract of *Euphorbia hirta*, 0.5 ml of Benedict"s reagent was added. The mixture was heated for 2 minutes on a boiling water bath.[10]

Barfoed's test: To 2 ml of methanolic extract of *Euphorbia hirta*about 2-3 ml of Barfoed's reagent. Mixed it well and boiled it for one minute in the water bath and allowed to stand for a few minutes.[10]

Test for Proteins:
Biuret test:2 ml of methanolic extract of *Euphorbia hirta*was treated with 1 drop of 2% copper sulphate solution. To this 1 ml of ethanol (95%) was added and followed by addition of excess of potassium hydroxide pellets.[11]

Millon's test: To 2 ml of methanolic extract of *Euphorbia hirta* few drops of Millon's reagent was added.[12]

Test for Aminoacids:
The methanolic extractof *Euphorbia hirta* was dissolved in 10 ml of distilled water and filtered through Whatmann No. 1 filter paper and the filtrate is subjected to test for Amino acids. [10]

Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of methanolic extractof *Euphorbia hirta*. [13]

Test for Flavonoids:
Shinoda test: Few magnesium chips were added to 2 ml of the methanolic extract of *Euphorbia hirta*and then 2 drops of dilute hydrochloric acid was added and warmed.[14]

Alkaline test: The methanolic extract of *Euphorbia hirta* was treated with 10% ammonium hydroxide solution.[10]

Test for Poly Phenols:
Ferric chloride test: The methanolic extract of *Euphorbia hirta* was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added.[15]

Lead acetate test: The methanolic extract of *Euphorbia hirta*was dissolved in of distilled water and to this 3 ml of 10% lead acetate solution was added.[10]

Bromine water test: Three drops of bromine water were added to 2 ml of methanolic extract of *Euphorbia hirta*.[14]

Test for Glycosides:
Borntrager's test: To 2 ml of methanolic extract of *Euphorbia hirta*, 3 ml of choloroform was added and shaken, then choloroform layer is separated and 10% ammomia solution was added to it.[16]

Keller-killiani test:0.5 ml of methanolicextract of *Euphorbia hirta*was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1ml of concentrated sulphuric acid.[14]

Test for Alkaloids:
Dragendorff's: To 2 ml of methanolicextract of *Euphorbia hirta*in test tube, 1 ml of dragendoff'sreagent was added drop by drop.[14]

Mayer's test: To a few ml of methanolic extract of *Euphorbia hirta*, two drops of Mayer's reagent was added along the sides of test tube.[16]

Wagner's test: A few drops of Wagner's reagent was added to few ml of methanolic extract of *Euphorbia hirta*along the sides of test tube.[17]

Hager's test: To 2 ml of methanolic extract of *Euphorbia hirta*, two drops of Hager's reagent was added.[18]

Test for Steroids-Terpenoids:
Salkowski Reaction: To 2 ml of methanolic extract of *Euphorbia hirta* in test tube, 3 drops of concentrated sulphuric acid was added to form a lower layer.[14]

Liebermann-Buchard reaction: The methanolic extract of *Euphorbia hirta* was dissolved in 2 ml acetic anhydride. To this, 2 drops of concentrated sulphuric acid was added slowly along the sides of the test tube.[19]

Results and discussion:
Pharmacognostic Analysis:
The pharmacognostic studies revealed the physicochemical parameters of the selected plant powder like foreign organic matter, alcohol soluble extractive, water soluble extractive, pH, Loss on drying, ash content and acid insoluble ash as shown in table 1 - which might help us in assessing its quality.

Table 1:Physicochemical parameters of powdered *Euphorbia hirta*s leaves.

Parameter	IE
Water soluble extractive	26.25% w/w
Alcohol soluble extractive	33.45%
Loss on drying	4.59%
Ash content	4.41%
Acid insoluble ash	0.57%
Foreign organic matter	2.48%
pH 1%w/v	5.24

Organoleptic characters:
The organoleptic characters of the methanolic extract of the plant are shown in table – 2.

Table 2: Organoleptic characters of methanolic extracts of Euphorbia hirtas leaves.

Parameter	IE
Colour	green
Odour	characteristic
Taste	characteristic
Physical appearance	Free flowing powder

Phytochemical Analysis:
The phytochemical analysis showed presence of carbohydrates, flavonoids, alkaloids, steroids and triterpenoids as shown in table – 3. The presence of above constituents in the plant extracts alone or in combination might be responsible to

exhibit its pharmacological activity. Hence, the further pharmacological screening and application of modern scientific technology might help us to derive therapeutically active compounds.

Table 3: PHYTOCHEMICAL TEST OF METHANOLIC EXTRACTS OF EUPHORBIA HIRTALEAVES.

Test	Result
Test for Carbohydrates:	
a) Molisch's test	+
b) Fehling's test	+
c) Benedict's test	+
d) Barfoed's test	+
Test for Proteins:	
a) Biuret test	_
b) Millon's test	_
c) Test for Aminoacids:	
a) Ninhydrin test	_
Test for Flavonoids:	
a) Shinoda test	+
b) Alkaline test	+
Test for Polyphenols:	
a) Ferric chloride test	_
b) Lead acetate test	_
c) Bromine water test	_
Test for Glycosides:	
a) Borntrager's test	-
b) Keller-killiani test	-
Test for Alkaloids:	
a) Dragendorff's	+
b) Mayer's test	+
c) Wagner's test	+
d) Hager's test	+
Test for Steroids-Terpenoids:	
a) Salkowski Reaction	+
b) Liebermann-Buchard reaction	+

*(-): Absent, (+): Present.

Summary and conclusion:

Ayurveda is one of the traditional systems of medicine practiced in India and Sri Lanka and can be traced back to 6000 B.C.Synthetic drugs available in market are found to have side effects so to overcome this problem more natural compounds with potent therapeutic value with different activities like anti-inflammatory,hepatoprotective, antimicrobial and immunomodulatory etc., should be developed. Hence, intense research should be carried out in plant medicine to establish its pharmacognostic and phytochemical profile, its efficacy and safety. In the present study, the selected leaves of *Euphorbia hirta*was collected, dried and subjected to size reduction to get uniform coarse powder. The powdered material of plant was then subjected to pharmacognostic studies to determine its physicochemical parameters. The pharmacognostic studies revealed the important parameters of the plant extract like foreign organic matter, alcohol soluble extractive, water soluble extractive, pH, Loss on drying, ash content and acid insoluble ash.Then the powdered plant material wassubjected to cold extraction i.e. maceration at room temperature with methanol as solvent. The methanolic extract of *Euphorbia hirta*s leaves were dried at reduced temperature using rotary evaporator. The dried extract was then subjected to organoleptic and phytochemical analysis. The methanolic extract of *Euphorbia hirta*sleaves showed presence of alkaloids, carbohydrates, flavonoids, triterpenoids and steroids. Hence, the present study confirms the presence of diverse group of compounds and might prove as rich source of therapeutic value. Extensive study might provide medicinally active compounds.

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